#31
Linda

Docket No. 2016-4005US1 9/17/03

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Blake, M. et al.

Group Art Unit: 1645

Serial No.: 09/207,188

Examiner: S. Devi

Filed: December 8, 1998

For: GROUP A STREPTOCOCCAL POLYSACCHARIDE IMMUNOGENIC COMPOSITIONS AND METHODS

Mail Stop AF
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF FRANCIS J. MICHON, PH.D.
UNDER 37 C.F.R. § 1.132

Sir:

I, the undersigned, Francis J. Michon, declare and state that:

1. I am an inventor of the above-identified application.

2. I am an expert in the area of carbohydrate chemistry. My education and professional experience are set forth on the attached copy of my Curriculum Vitae (Exhibit 1).

3. I am presently employed as Senior Director of Carbohydrate Chemistry by Baxter Hyland Immuno, a division of Baxter Healthcare, Inc. Prior to my employment by Baxter Hyland Immuno, I was employed by North American Vaccine, Inc. which was subsequently acquired by Baxter Healthcare, Inc.

4. As stated in my Curriculum Vitae, my area of expert training and experience is carbohydrate chemistry, preparation of polysaccharides from biological materials, and the use of such polysaccharides conjugated to proteins and the use of such conjugates in vaccines.

5. I have read and understand U.S. Serial No. 09/207,188, including the claims. I have also reviewed the Office Action dated April 9, 2003 concerning the above-identified patent application, and in particular the Examiner's contention that claims 80, 81, and 83-93 are not enabled with regard to the scope, because the Examiner contends that "there is neither any showing, nor is it predictable that one skilled in the art can reproducibly and successfully practice the claimed method using a formula I polysaccharide-protein conjugate or a formula I polysaccharide-protein conjugate wherein n is 3 to 50" (*See*, Office Action, pg. 6).

6. I am aware that application U.S. Serial No. 09/207,188 describes Group A streptococcal polysaccharide immunogenic compositions and claims methods of administering a polysaccharide-protein conjugate or polysaccharide-protein fragment conjugate for eliciting protective antibodies specific to group A streptococcal polysaccharide.

7. I am further aware that the Examiner rejected claim 80, 81, and 83-93 under 35 U.S.C. §112, first paragraph for lack of enablement. I respectfully disagree with these rejections. For the reasons described below, I believe that the specification adequately discloses to one skilled in the art how to make and use the conjugates according to the claimed methods. Moreover, based on our surprising finding disclosed in the specification that antibodies to Group A streptococcus confer resistance to infection, paragraph page bridging pages 19-20, and the disclosure in Example 7 of high antibody titers in response to immunization with conjugates, our assertion that such conjugates would be useful as vaccines to confer protection against infection of Group A streptococcus are highly

credible. In addition, subsequent to the filing of the application, I participated in further experiments which confirmed the efficacy of the Group A streptococcal protein conjugates and which were reported in Sabharwal, et al. presented at the Proceedings of the XIV Lancefield International Symposium on Streptococci and Streptococcal Disease, Auckland, New Zealand, 1999. October 11-15. (in press). (Exhibit 2).

8. I, Francis Michon, together with Hemant Sabharwal, Milan Blake, and John B. Zabriskie, co-authored the paper entitled, "Immunization with Group A Streptococcal Carbohydrate Protects Against Group A Streptococcal Infections in Mice" [Sabharwal, et al. presented at the Proceedings of the XIV Lancefield International Symposium on Streptococci and Streptococcal Disease, Auckland, New Zealand, 1999. October 11-15. (in press)]. The Sabharwal, et al. paper reports the results of passive and active immunization with a Group A streptococcal carbohydrate and conjugate.

a. The bacterial strains reported in the Sabharwal, et al. publication were S42/46 (M Type 6), D58/93/7 (M Type 3), and S23 (M Type 14) [Rockefeller University Collection]. The strain to be tested was suspended in 5 ml of Todd-Hewitt (TH) broth and grown for 18 hours at 37 °C. The overnight culture was diluted 1:3 with fresh pre-warmed TH broth and incubated for approximately 2 hours at 37 °C or until the optical density (OD) reached a 0.73 reading at 600 nm. The culture was then appropriately diluted to deliver sufficient numbers of Group A streptococci to cause 100% mortality in the control mice over a 72 hour time period. Injections were given i.p. in a volume of 200 microliters. Samples (20 microliters and 100 microliters) of the sample dilutions were placed in petri dishes to which 10 ml of brain heart infusion agar (2.4%) containing 0.5% defibrinated sheep blood

was added. The plates were incubated overnight at 37 °C and the colonies counted the next day.

b. In the Sabharwal, et al. publication, passive protection studies were performed to determine whether Group A Streptococcal carbohydrate antibodies could protect against a lethal challenge of live Group A streptococci. Rabbits were immunized with Group A carbohydrate ("CHO") conjugated to tetanus toxoid at a dose of 500 micrograms in four separate sites. The conjugate was in Complete Freund's adjuvant. A second dose was administered 3 weeks later in incomplete Freud's adjuvant. Animals were bled two weeks later. The CHO antibody titers were then assayed. Animals were boosted subcutaneously with the same dose mixed with incomplete adjuvant until titers of $1x10^6$ were reached. Immunoglobulin (IgG) fractions were then collected from immunized rabbits and used for passive immunity studies. One hour before murine challenge with Group A streptococcal bacteria, 0.3 mls of the rabbit antibody (diluted 1:5 or 1:10) or normal rabbit serum (NRS) was injected into the test mice intraperitoneally (i.p).

c. The Sabharwal, et al. publication also reports active immunization studies to determine whether active immunization of mice using Group A streptococcal carbohydrate conjugated to tetanus toxoid could protect against a similar lethal challenge model. Mice were injected subcutaneously with Group A carbohydrate conjugated to tetanus toxoid at a concentration of 5 micrograms (200 microliters) per dose mixed with alum adjuvant. A second dose was administered 4 weeks later and repeated for a total of 4 doses. Animals were checked for Group A streptococcal carbohydrate titers by retro-orbital bleeding 10 days after the last dose. Control mice were injected in the same manner with adjuvant and tetanus toxoid alone without the addition of CHO. Animals were then challenged i.p. with live Group A streptococci as described above.

9. Sabharwal, et al. report that group A streptococcal carbohydrate conjugates can protect animals against a lethal challenge with two different M+ type specific group A streptococcal strains. Sabharwal, et al. describe the use of such conjugates to elicit both passive and active protection.

10. The passive protection and active immunization studies have the following results:

Table 1. Passive protection test in mice against Group A Streptococcus Type 6 (S43/46).

Serum	Colonies	Mice*
NRS	200-500	3/26**
Group A CHO Ab	200-500	16/26

*Number of Mice Survived/Injected
**p<0.001

Table 2. Passive protection test in mice against Group A Streptococcus Type 3 (D58/93/7).

Serum	Colonies	Mice*
NRS	$1.7\text{-}4.6 \times 10^5$	3/15**
Group A CHO Ab	$1.7\text{-}4.6 \times 10^5$	13/15

*Number of Mice Survived/Injected
**p<0.041

Table 3. Active immunization studies with Group A Streptococcal CHO in mice challenged with live Type 6 (S43/46) Streptococci.

Group	Adjuvant	Inoculum Range	Survived/Injected
CHO-TT Conjugate	Alum	$3\text{-}7 \times 10^5$	11/15*
TT	Alum	$3\text{-}7 \times 10^5$	2/15*

*p = 0.003

Table 4. Active immunization studies with Group A Streptococcal CHO in mice challenged with live type 14 (S23) Streptococci.

Group	Adjuvant	Inoculum Range	Survived/Injected
CHO-TT Conjugate	Alum	3-3.6 X 10^6	18/23*
TT	Alum	3-3.6 X 10^6	5/22*

*$p<0.001$

11. Tables 1 and 2 clearly demonstrate that rabbit antibodies raised against Group A streptococcal carbohydrate (CHO) conjugated to tetanus toxoid (TT) protects against a lethal challenge with two M+ type specific streptococcal strains. In fact, there was a statistically significant difference in the ratio of the number of mice that survived to total number of mice injected with Group A Streptococcal CHO-TT rabbit antibody compared to control as shown in Tables 1 and 2. Furthermore, Tables 3 and 4 show that in the active immunization studies, immunization with Group A Streptococcal CHO conjugated to tetanus toxoid also protected animals challenged with live type 6 and type 14 Streptococci. There is a statistically significant decrease in the number of deaths in immunized mice when compared to control.

12. Sabharwal, et al. report that passive administration of Group A Streptococcal CHO antibodies and active immunization of animals protect against a lethal challenge of Group A Streptocci. These results strongly support the use of Group A streptococcal carbohydrates as a vaccine to prevent Group A streptococcal infections. In particular, since the Sabharwal, et al. publication reports that the Group A streptococcal carbohydrate conjugated to tetanus toxoid with alum adjuvant elicited

protective antibodies and that the Group A streptococcal carbohydrate antibodies injected into mice provided passive protection against live Group A streptococci, one skilled in the art would understand from reading Sabharwal, et al. that the protective antibodies elicited by injecting Group A streptococcal conjugates provide protection against Group A streptococcal infection supports the claimed methods of the present invention. It is within the scope of the skilled artisan to correlate the methods of eliciting protective antibodies using a Group A streptococcal conjugate to the claimed method of the present application (USSN: 09/207,188).

13. Thus, the present application 09/207,188 describes, and the Sabharwal, et al. publication confirms a method of eliciting group A streptococcal protective antibodies by administering polysaccharide-protein conjugates. The patent application clearly describes and enables one skilled in the art to use a polysaccharide-protein conjugate having formula (I) for eliciting protective antibodies against group A streptococci. It is clear to the skilled artisan from the description in the specification and the Sabharwal, et al. publication that the polysaccharide-protein conjugate is useful and enabled in eliciting group A streptococcal protective antibodies in animals immunized with the conjugate. Furthermore, one skilled in the art would be capable of reproducibly and successfully practicing the claimed method using a formula I polysaccharide-protein conjugate or a formula I polysaccharide-protein conjugate where n is 3 to 50 without undue experimentation.

14. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such

willful false statements may jeopardize the validity of the application of any patent issuing thereon.

Date: 08 Sept. 03

Francis J. Michon, Ph.D.